Reprogramming of Human Hair Follicle Dermal Papilla Cells into Induced Pluripotent Stem Cells

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TO THE EDITOR

The derivation of engineered stem cells or human induced pluripotent stem cells (iPSCs; Takahashi *et al.*, 2007) through the reprogramming of adult fibroblasts was a major advancement in the field of cell therapeutics and regenerative medicine. Introduction of defined transcription factors in various combinations (cMYC, SOX2, OCT4, KLF4, NANOG, LIN28) has subsequently been used to reprogram different somatic cell types, including patient-specific cells, into iPSCs, with subsequent directed differentiation into various lineages (Kiskinis and Eggan, 2010). For example, our previous studies defined conditions under which keratinocytes can be generated from iPSC (Itoh *et al.*, 2011).

Despite recent advances, there are still major challenges associated with using conventional iPSCs for clinical applications, for example, reprogramming efficiency is low and viral integration frequently occurs. To facilitate iPSC generation, synthetic small molecules and modified mRNAs are now being used to replace transcription factors during reprogramming (Zhu *et al.*, 2010; Warren *et al.*, 2011). The choice of cell type also has a significant effect, e.g., umbilical vein endothelial cells and amniotic fluid–derived cells possess higher reprogramming efficiencies when four transcription factors are used (Li *et al.*, 2009; Galende *et al.*, 2010; Panopoulos *et al.*, 2011). Comparatively, human fetal neural cells have a low reprogramming efficiency, but can be reprogrammed using just one factor, OCT4 (Kim *et al.*, 2009). However, accessibility problems render the use of fetal cells undesirable. Skin keratinocytes, in contrast, are highly accessible, and have been reported as being 100 times more efficient than fibroblasts at reprogramming (Aasen and Belmonte, 2010).

Here, we assessed the reprogramming potential of another accessible cell type located within the skin, the hair

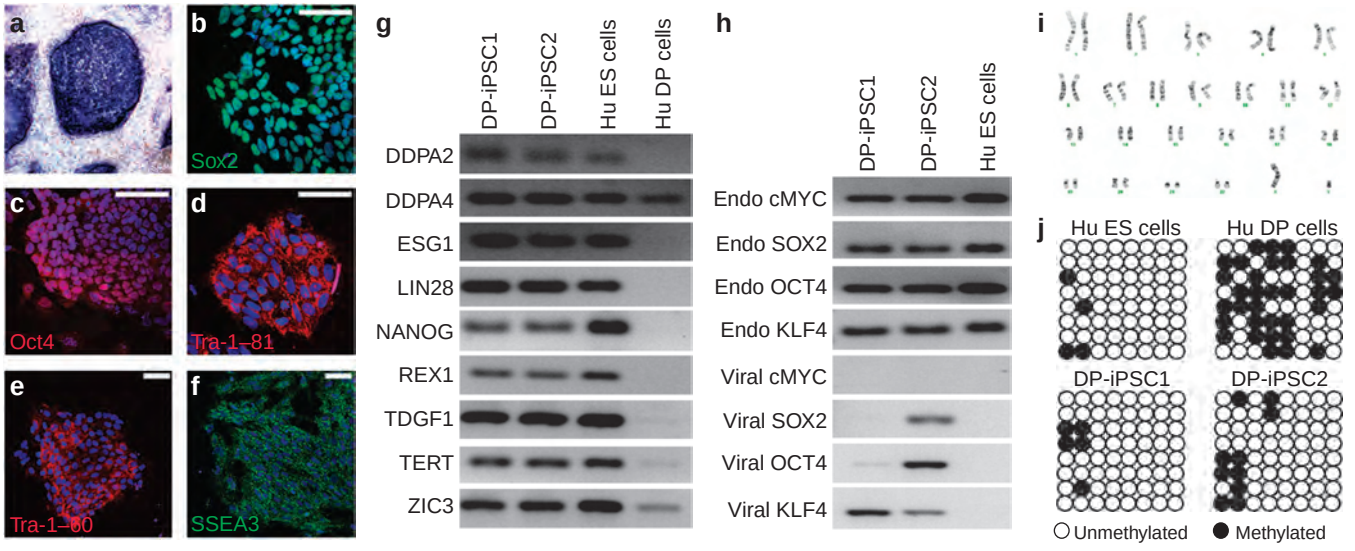

Figure 1. Pluripotency and characterization of DP-iPSCs cell lines. (**a**) Alkaline phosphatase was observed in DP-iPSC colonies after formation. After expansion, the transcription factors (**b**) Sox2 and (**c**) Oct4 were detected by immunofluorescence. In addition, ESC pluripotency markers (**d**) tumor-related antigen 1–81, (**e**) tumor-related antigen 1–60, and (**f**) stage-specific embryonic antigen 3 were detected on cell surfaces. (**g**) ESC pluripotency markers were detected by reverse transcription–PCR (RT–PCR) in two lines of DP-iPSCs, and in human ESCs, although the majority were not detected within cultured human DP cells. (**h**) RT–PCR of endogenous and viral transcription factors indicated that both DP-iPSC lines under analysis expressed endogenous cMYC, SOX2, OCT4, and KLF4, whereas viral silencing was more random. (**i**) Karyotype analysis of the DP-iPSCs indicated a normal karyotype of 46XY. (**j**) Methylation analysis of the NANOG promoter showed a repressed promoter in cultured DP cells, but an active promoter in ESCs and DP-iPSCs. Bar = 100 μm.
DP, dermal papilla; ESC, embryonic stem cell; Hu, human; iPSC, induced pluripotent stem cell.

Abbreviations: DP, dermal papilla; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell

follicle dermal papilla (DP), to determine whether they also had an increased reprogramming efficiency. We focused on the DP, located at the base of the hair follicle, since it is an enriched stem cell niche of clinical interest in other contexts (Hunt *et al.*, 2008; Biernaskie *et al.*, 2009). When we analyzed the endogenous levels of the transcription factors commonly used in reprogramming, we found high levels of endogenous *SOX2* and *NANOG* in intact human DP (Supplementary Figure S1 online). Immunofluorescence analysis of SOX2 confirmed these observations, but when DP cells were propagated in culture they no longer expressed these pluripotency markers. In this regard, the DP cells appeared similar to cultured interfollicular fibroblasts, however, cultured DP cells are distinct from fibroblasts insofar as they demonstrate remarkable plasticity differentiating down multiple mesenchymal lineages in culture (Richardson *et al.*, 2005).

To assess the reprogramming capacity of human DP cells, we used pMX-based retroviruses using varying combinations of four transcription factors—cMYC, SOX2, OCT4, and KLF4 (see Supplementary Materials and Methods online)—to transduce DP cells at passage 3. We observed iPSC colony formation 28 days after transduction when we used four factors, resulting in a reprogramming efficiency of ~0.02%. However, we were unable to generate any iPSC colonies using less than four factors, in any combination, from cultured human DP. Our colonies appeared to be similar to embryonic stem cells (ESCs) in their appearance, exhibiting a high nuclear/cytoplasmic ratio and expressing alkaline phosphatase (Figure 1a). After colony expansion, we observed ESC markers (Supplementary Table S1 online) localized within DP-iPSC colonies using immunofluorescence (Figure 1b–f). In addition, reverse transcription–PCR analysis against ESC genes (Supplementary Table S2 online) indicated expression within our DP-iPSCs but not within cultured DP cells, indicative of successful reprogramming (Figure 1g). Reverse transcription–PCR of the reprogramming factors in their endogenous and viral forms also showed that although DP-iPSCs express all four factors endogenously, there is random viral silencing after DP-iPSC expansion (Figure 1h). Despite this, the karyotype of our

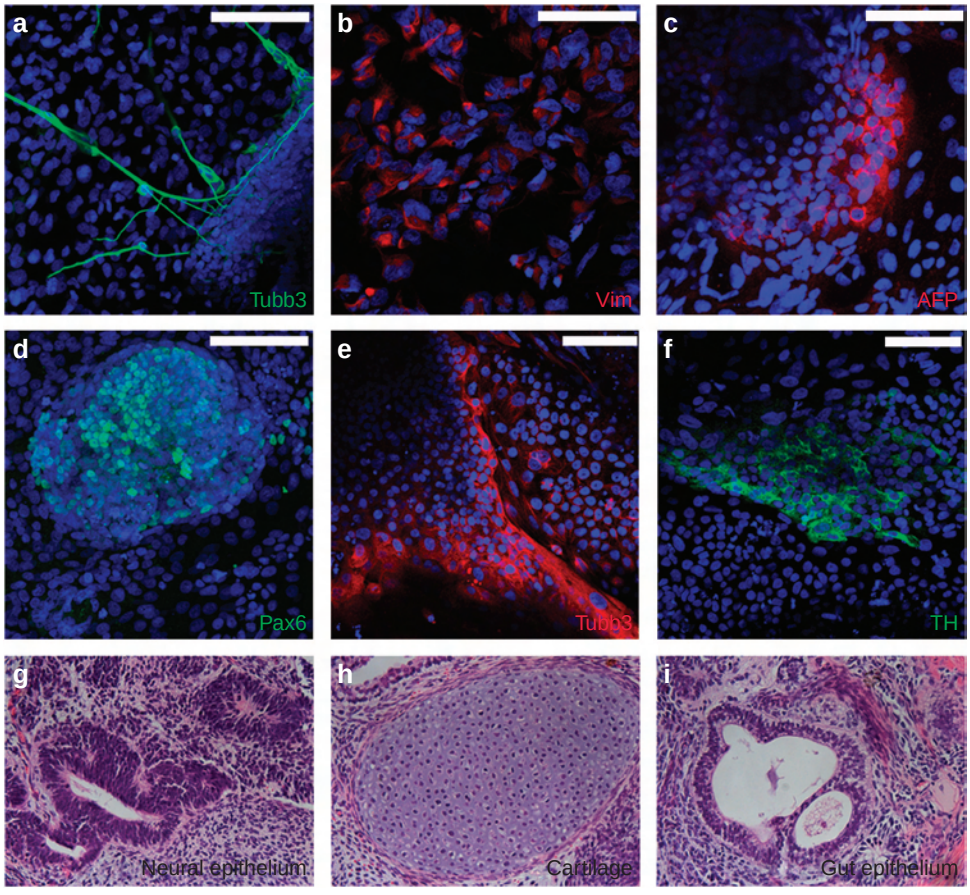

Figure 2. Differentiation of DP-iPSCs. After *in vitro* differentiation of embryoid bodies of DP-iPSCs, immunofluorescence analysis showed expression of markers characteristic of (**a**) ectoderm (βIII-tubulin), (**b**) mesoderm (vimentin), and (**c**) endoderm (α-fetoprotein). DP-iPSCs were differentiated on PA6 feeder layers down a dopaminergic neuron lineage, and after 14 days expressed neuronal markers (**d**) paired box 6, (**e**) βIII-tubulin, and (**f**) the dopaminergic neuronal marker tyrosine hydroxylase. After *in vivo* formation of teratomas, hematoxylin and eosin staining revealed that DP-iPSCs had differentiated down (**g**) ectodermal, (**h**) mesodermal, and (**i**) endodermal lineages. Bar = 100 μm. DP, dermal papilla; iPSC, induced pluripotent stem cell.

DP-iPSCs remained normal after extended culture (Figure 1i). We also performed bisulfite sequencing of the *NANOG* promoter, and found that similar to ESCs, DP-iPSCs have an unmethylated promoter, indicating a transcriptionally active *NANOG* status (Figure 1j). Comparatively, the *NANOG* promoter in cultured DP cells is methylated, and therefore silenced.

To interrogate the pluripotency of our DP-iPSCs, we used both *in vitro* and *in vivo* techniques. First, we used DP-iPSCs to generate embryoid bodies, which spontaneously differentiated into ectodermal, mesodermal, and endodermal lineages (Figure 2a–c). We also successfully directed our DP-iPSCs to differentiate into a neuronal lineage, which expressed various neuronal markers, including the dopaminergic neuron marker tyrosine hydroxylase (Figure 2d–f). For *in vivo* differentiation analysis, we injected DP-iPSCs subcutaneously into athymic mice, where they formed teratomas containing tissues derived from all three germ lineages (Figure 2g–i).

In this study, we sought to determine whether human DP cells may represent a reprogrammable cell type for use in generating iPSCs. We were able to successfully reprogram human hair follicle DP cells into DP-iPSCs, and showed their similarity to ESCs. Similar to ESCs, DP-iPSCs also exhibit pluripotency and can differentiate into various cell and tissue types *in vitro* and *in vivo*. We conclude that DP cells are indeed reprogrammable, however, their reprogramming efficiency is not significantly different from adult human fibroblasts (Takahashi *et al.*, 2007). Thus, unlike keratinocytes, there is not a significant improvement in reprogramming efficiency using DP cells rather than dermal fibroblasts when making human iPSCs. This is in contrast to recent reports demonstrating that mouse DP cells have a significant reprogramming advantage over mouse fibroblasts, and are capable of being reprogrammed using Oct4 alone (Tsai *et al.*, 2011).

One explanation for this difference is the requirement to expand human DP cells with an explant method (Ohyama *et al.*, 2010), which leads to a loss of SOX2 and NANOG expression (Supplementary Figure S1 online). In contrast, mouse DP cells can be enzymatically dissociated from intact tissues and transduced shortly after, allowing for retention of high Sox2 expression (Tsai *et al.*, 2011), which may partly confer the reprogramming advantage. Perhaps a more efficient way of making human iPSCs would be through reprogramming SKPs, of which DP cells are an enriched source (Hunt *et al.*, 2008). Our work underscores the necessity of conducting studies using human cells in addition to mouse, in order to specifically determine their potential benefit to regenerative medicine. We conclude that the human hair follicle provides an accessible source of reprogrammable cells for making iPSCs, both in the form of outer root sheath cells that can be isolated from a single plucked hair fiber, as previously shown (Aasen and Belmonte, 2010), and here, for the first time, using cells derived from the DP.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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